Blood Lipids and Lipoproteins:

Quantitation, Composition, and Metabolism

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EDITED BY

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Preface

The subject of blood lipids and lipoproteins covers a broad area of scientific knowledge that is not easily separated into the well-defined categories found in other scientific fields that are less interdisciplinary in character. The investigators interested in blood lipids are a heterogeneous group, ranging from theoretical biophysicists and structural organic chemists through biochemists and physiologists to practicing physicians. Consequently reports of original research in this field are scattered throughout the chemical and biomedical literature, making it difficult for even the specialist to retrieve significant information. And although there are a number of books on lipoproteins, as well as discussions of cellular lipids in several books on the erythrocyte, this problem is compounded by the lack of a modern, comprehensive treatise devoted exclusively to blood lipids and lipoproteins. This book attempts to fill this gap by providing in a single volume a reasonably complete summary of the field. Obviously it is not possible to cover thoroughly in a single volume all aspects of any scientific subject. Blood lipids and lipoproteins are certainly no exception. Yet I hope that the reader will find between the covers of this book adequate coverage of the overall subject without recourse to a full library, and at the same time adequate guidance to additional and more specialized information.

The authors were asked to treat their subject matter critically and objectively, but not to feel obligated to remove all signs of their own preferences and specialized interests. Thus each chapter reflects, more or less, the area of competence and specialization of its authors. No requirement was made to cite every publication or random hypothesis that has appeared in the last 50 years. Obviously, no matter how impartial an author tries to be, he brings a subjective bias to his subject matter. But this in itself can be useful. The reader can compare the chapters in this volume against those in past and future publications by others, thus obtaining a more balanced view of the subject than he can gain from the work of a single author.

Another reason for adding this book to the already prodigious number of scientific treatises currently available is my concern that an arbitrary segregation has occurred in the study of blood lipids, probably more through historical accident than through any deliberate actions on the part

of the scientific community. Frequently the lipids and liproproteins of plasma and serum are treated separately from those of the cellular components of blood, and interactions between them are largely ignored. However, since it has been known for two decades that cholesterol is rapidly exchanged between erythrocytes and plasma, the justification for this type of treatment is questionable. More recently work on the plasma lecithin: cholesterol acyltransferase reaction has shown that lipids other than cholesterol also interact between cells and plasma. In addition, current studies on blood lipids in various diseases—such as acanthocytosis, hepatitis, and several anemias—have demonstrated that there is an intimate and vital relationship between the lipids of blood cells and plasma. Unquestionably, as we learn more about the structure and function of the lipids and lipoproteins of plasma and cells, their direct interrelationship with, and dependence on, one another will become even more manifest.

In any event the evidence is now overwhelming that lipids of both plasma and cells should be regarded as different facets of a single entity and that investigators should consider the possible consequences of lipid interactions between the two compartments regardless of their own specialized interests. My hope is that this volume will be a step in this direction.

I wish to acknowledge my gratitude to Dr. George Rouser for his assistance in the preliminary phases of the preparation of this volume and Dr. Valeska Evertsbusch for copy editing and reorganizing the tables in some of the chapters. To the authors of this volume I am grateful not only for their contributions but also for their understanding and patience during the preparation and production of this book. My thanks are also due to numerous publishers for permission to reproduce many of the figures and tables used in this volume.

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Livermore, California
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1. Handling, Extraction, and Storage of Blood Samples

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I. INTRODUCTION

The withdrawal of blood for scientific purposes is as old as experimental biology. Early methods were crude and often involved simply cutting the skin or vein of the experimental subject (1). Blood sampling did not reach technical excellence until the advent of the centrifuge and anticoagulants in the early twentieth century (2). The phenomenon of clotting did not prevent some early studies on blood, particularly since serum can

be recovered after blood has been allowed to clot for several hours (3). Early analyses of blood lipids were primarily qualitative (4, 5).

The pioneering work of Bloor (6, 7) marks the real beginning of the quantitative biochemistry of blood lipids. The difficulties encountered by early investigators are still major pitfalls today, and modern investigators should be cognizant of them. Briefly, they involve the coagulation of blood, contamination of serum with erythrocytes and/or platelets and leukocytes (and vice versa), and denaturation and autoxidation of lipids during isolation and storage. With due diligence and proper precautions, these problems can be avoided without inconvenience to the investigator. This chapter outlines procedures that, in the author's opinion, so facilitate the processing of blood samples that the experimental results are as accurate as the analytical procedures and are uninfluenced by sampling techniques. No attempt is made to present a historical development or to cite every technique reported in the literature.

II. DRAWING BLOOD SAMPLES

A. Preparation of Blood Containers

Small samples (approximately 1–25 ml) of blood can be drawn directly into evacuated centrifuge tubes. The tubes can be made of either glass or plastic and should be clean and sterile. Siliconizing, a useful technique in many procedures because it prevents the coagulation and cell fragmentation resulting from contact with glass surfaces (8), should be avoided when lipids are being analyzed, because silicone oil is soluble in organic solvents (9) and can be carried through the analytical procedures. A drop of heparin solution or a few crystals of heparin can be added to the tubes to prevent coagulation. The tubes should be shaken gently during the drawing of blood to distribute the anticoagulant uniformly throughout the sample. Without thorough mixing, coagulation may take place in the upper layers of the sample; this is particularly troublesome when large samples are drawn.

Samples of less than 20-ml total volume are often drawn directly into syringes (10), to which the anticoagulant can be added before the sample is drawn. Of course the sample must be transferred from the syringe to the centrifuge tube soon after sampling. Direct syringe sampling is also the method of choice for sampling the blood of small animals, since only small volumes can be obtained.

Blood bottles or plastic blood bags are the preferred containers for samples in the range of 50 to 500 ml. Within the last few years the plastic

blood bag has almost entirely replaced the glass blood-storage bottle (11). However, hemolysis remains a problem with large-volume samples, even when plastic blood bags are used. The plastic blood bag need not be siliconized (11) and is unbreakable. However, it cannot be evacuated, and this may decrease the rate at which the sample can be drawn.

Methods for obtaining large samples of blood—for example, when a large animal is to be exsanguinated—cannot be standardized. Drawing the blood into large Erlenmeyer flasks is one method commonly used in the author's laboratory. Buckets and large beakers can also be used.

Anticoagulants are not always necessary (12). If only serum is needed, the blood is drawn and allowed to clot before the cells are separated from the serum. This simplifies the removal of the cells and, more important, removes the fibrinogen from the serum. This procedure has had noticeable success in studies of human-lipoprotein patterns (13) and in large-scale screening studies on the atherogenicity of lipoprotein levels (14) as well as in biophysical lipoprotein research (15). However, the procedure promotes the activity of plasma cholesterol acyltransesterase, which alters the serum-lipid patterns for cholesterol–cholesterol ester and lecithin–lysolecithin, as pointed out originally by Sperry (16) and more intensively studied by Glomset (17) (see Chapter 14).

Several anticoagulants are available to prevent clotting and the destruction of erythrocytes during blood sampling. Isotonic citrate dextrose solution, a good choice when whole blood must be preserved for metabolic studies or reused, as in infusion experiments (18, 19), has the disadvantages that it dilutes the blood and significantly increases the volume. This is particularly annoying when large amounts of blood are processed in the laboratory. The preferred anticoagulants are ethylenediaminetetraacetic acid (EDTA) and heparin (20–22). In the author's experience heparin in solution is a convenient anticoagulant, having little or no effect on the blood lipids. Ethylenediaminetetraacetic acid is also a reliable anticoagulant; moreover, it sequesters the divalent metallic ions that promote the autoxidation of unsaturated fatty acids in blood lipids (23).

The use of antioxidants is also recommended. Initially the best way of avoiding autoxidation, which may be promoted enzymatically in drawn whole blood, is to cool the blood immediately after, or even during, the sampling process. Centrifuge tubes can be prechilled in an ice bath and immediately returned to the bath after receiving the sample. Plastic blood bags can be immersed in an ice bath even as the sample is drawn. The addition of such antioxidants as butylated hydroxytoluene (BHT) or tocopherol to fresh blood is not necessary when blood is kept below 10°C until processing, provided that processing is not unduly delayed. This is

particularly true if EDTA is present. Antioxidants should be added to blood-lipid samples during lipid extraction by addition to the extracting solvents.

B. Methods of Sampling Animal Blood

The choice of sampling method depends on the size of the experimental animal and on the size of the desired sample. Sampling from human subjects presents a special case and is usually handled in clinics or hospitals. Procedures for sampling the blood of animals fall into two categories: (a) those used for small animals (e.g., rats, guinea pigs, and mice) and (b) those used for any larger animals.

Blood is often obtained from small animals by sacrificing and exsanguinating them. Heart puncture recovers much of the total blood from a rat or mouse (24). Small samples can be drawn from rats or mice by slicing off the tip of the tail and collecting the blood (25), but this method yields total volumes of less than 1 ml; alternatively hypodermic needles can be inserted into the veins of small rodents (26–28). For specific techniques and information on blood volumes in small animals the reader is referred to more specialized treatises (29). Animals weighing a few hundred grams or less have small blood volumes (30). Samples from several animals are usually pooled for lipid analysis because the amount of lipids present in the circulation is small (31). Ten milliliters is frequently the minimum needed for the chromatographic techniques described in other sections of this volume.

Small samples can be drawn from large animals by syringes without anesthesia (32, 33). The location of the puncture is chosen at the convenience of the investigator. The ear is a good site because the veins are visible under the epithelial layer (34). Dogs are often sampled in the veins of the hindlegs (34). Several specialized techniques and locations for drawing blood from large experimental animals have been reported; the reader is referred to texts on experimental physiology or veterinary medicine for details (24, 34, 35).

Venipuncture is the normal procedure for large animals, and the jugular vein is a convenient site (36). Large animals must be restrained or anesthetized (37). If blood is drawn from a specific internal site, it is necessary to use a catheterization procedure (38), which always requires anesthesia.

It is sometimes necessary to draw blood postmortem. Immediately after death blood can be drawn from experimental animals with no problems. If the animal has been dead for several hours, blood coagulation, along with other postmortem changes, often complicates or prevents meaningful